Polyamines: Metabolism, Regulation, and Functions in Crop Abiotic Stress Tolerance

19

Swati Sinha and Maneesh Mishra

Abstract

Polyamines are small, positively charged, organic compounds containing more than two amino groups. They are omnipresent in plants and produced during various metabolic processes. Environmental fluctuations owing to greenhouse gases, pollution, deforestation, and global warming are known to hamper plants' normal growth, production, and developmental processes causing various forms of abiotic stresses such as drought, salinity, heat, cold, osmolarity, etc. Polyamines are considered important to plants as they provide support in maintaining normal growth and development of plants even during stressed conditions. They play crucial role whether present at endogenous levels or fortified exogenously to plants utilizing plant tissue culture or genetic transformation techniques. Although they are regarded important for plants, still their mode of action and regulation during plant stress conditions is still not well understood. In this chapter, their endogenous production, mode of action and regulation is described at length so as to facilitate a broader and clearer picture to the researchers to understand the importance of them in combating various abiotic stresses in plants.

Keywords

Polyamines · Abiotic stress tolerance · Putrescine · Spermidine · Spermine

---

S. Sinha (✉) · M. Mishra
ICAR – Central Institute for Subtropical Horticulture, Lucknow, Uttar Pradesh, India

S. A. Ansari et al. (eds.), *Augmenting Crop Productivity in Stress Environment*,
https://doi.org/10.1007/978-981-16-6361-1_19

19.1 Introduction

Polyamines (PAs) are polycationic aliphatic amines. They are present in plants abundantly and take part in major plant growth and development processes. Spermidine (Spd), spermine (Spm) and putrescine (Put) are common polyamines apparently involved in plant responses to microbial symbionts that are critical for plant nutrition (El Ghachtoul et al. 1996), and are involved in molecular signaling events in interactions between plants and pathogens (Martin-Tanguy 1987). PAs are important for plant growth development and environmental stresses as well (Evans and Malmberg 1989; Galston and Kaur-Sawhney 1990). PAs are synthesized from the amino acids ornithine or arginine. Their capacities to neutralize acids and act as antioxidant agents—as well as their roles in membrane/cell wall stabilization—make them indispensable for normal functioning of cells. DNA has an overall negative charge due to phosphate group attached to it (Basu et al. 1990; Pohjanpelto and Höltta 1996), so PAs tend to bind with DNA, thereby providing stability to the DNA helix (Beigbeder 1995; Tassoni et al. 1996) and pectic polysaccharides (D'Oraci and Bagni 1987). They have been shown to be involved in protein phosphorylation (Ye et al. 1994), post-transcriptional modifications (Mehta et al. 1994), and conformational transition of DNA (Basu et al. 1990). There is direct evidence that they are essential for growth and development in prokaryotes and eukaryotes (Tabor and Tabor 1984; Heby and Persson 1990; Tiburcio et al. 1990; Slocum 1991). PAs are considered to be signaling molecules, especially in stress situations (Evans and Malmberg 1989; Galston and Kaur-Sawhney 1995).

Naturally occurring PAs play pivotal role in plant metabolism such as effects on the tolerance mechanism to abiotic stresses (Meloni et al. 2003; Zeid 2004; Jiuju and Shirong 2005; Duan et al. 2007; Roychoudhury et al. 2011).

Abiotic stresses such as soil salinity, cold, frost injury, drought, acidity, and heavy metal affect plant growth, development, and productivity. Since these conditions produce stress and result in extensive losses to many agriculturally important crops, they have been the main subject of intense research. The accumulation of some functional substances, such as compatible solute, protective proteins, and polyamines, is an important element of the physiological and biochemical response to the stressful conditions. Polyamines have been proposed as a new category of plant growth regulators which are active in physiological processes, such as embryogenesis, cell division, morphogenesis, and development (Bais and Ravishankar 2002; Liu et al. 2006a). Though the physiological significance of polyamines in stress is not thoroughly understood, much progress has been made. The focus in the present paper is on progress concerning the involvement and potential role of polyamines in plant responses to abiotic stresses.

19.2 Polyamine Biosynthesis and Metabolism in Plants

Polyamine biosynthesis in plants is documented. Put is produced either directly from ornithine-by-ornithine decarboxylase (ODC, EC 4.1.1.17) or indirectly from arginine-by-arginine decarboxylase (ADC, EC 4.1.1.19) with two intermediates, agmatine and *N*-carbamoyl putrescine, and two corresponding biosynthetic enzymes, agmatine iminohydrolase (EC 3.5.3.12) and *N*-carbamoylputrescine amidohydrolase (EC 3.5.1.53) (Malmberg et al. 1998; Martin-Tanguy 2001). Put is converted into Spd via spermidine synthase (SPDS, EC 2.5.1.16) with the addition of an aminopropyl moiety provided by decarboxylated *S*-adenosylmethionine (dcSAM), which is catalyzed by *S*-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) using *S*-adenosylmethionine (SAM) as the substrate. Similarly, Spm is produced from Spd via spermine synthase (SPMS, EC 2.5.1.22) with the same aminopropyl moiety rendered by dcSAM. Apart from biosynthesis, polyamine degradation plays a crucial role in cellular polyamine titers regulation, which is primarily ascribed to two amine oxidases, diamine oxidase (DAO, EC 1.4.3.6), and polyamine oxidase (PAO, EC 1.5.3.11). DAO catalyzes the oxidation of Put to give pyrroline, which is further metabolized to g-aminobutyric acid (Cona et al. 2006) and PAO, and acts as a catalyst in the conversion of Spd and Spm to pyrroline and 1-(3 aminopropyl)-pyrroline, respectively, along with 1,3-diaminopropane in plants (Martin-Tanguy 2001; Šebela et al. 2001).

19.3 Polyamines Transport in Plants

It is estimated that PAs can be transported in long distances; the presence of large amounts of PAs has been observed in exudates of xylem and phloem sap; the vacuolar membrane had the highest capacity to transport them (Vladimir and Shevyakova 2007).

As per a study carried out in carrot cells, PAs (Put and Spd) flow through the cell by a transmembrane electrochemical gradient. Other research with maize roots, whose application of Put was performed exogenously, indicated that Put is transported through the plasma membrane by a process regulated by a protein carrier (Kusano et al. 2008). This carrier TPO1 of PAs is mainly located in vacuolar membrane and plasma membrane. Among the four PA carriers, those which are encoded by TPO2 and TPO3 are specific for Spm, while for Put, Spd and Spm are encoded by TPO1 and TPO4 (Uemura et al. 2005). TPO1 is dependent of pH (Uemura et al. 2005); in some research with lichen (*Evernia prunastri*), it was shown that the uptake of PAs depends on this variable (Kakkar et al. 1998). Uptake of Spr and Spd is performed at alkaline pH ($\mathrm{pH} = 8.0$), while inhibition of only Spd was observed at acidic pH ($\mathrm{pH} = 5.0$), next to the pH of the vacuoles found internally. This suggests that TPO1 carrier acts as a catalyst for polyamine excretion at acidic pH (Uemura et al. 2005). Put uptake occurred under different conditions of concentration and pH in African violet petals (*Saint pauliaionantha*), at a relatively lower concentration gradient (0.5–1.1 micromoles, $\mathrm{pH} = 5.0–5.5$) and to a high concentration gradient (from 0.1 to 100 millimolar, pH = 8.0) (Kakkar et al. 1998); therefore, PAs show the ability as buffers or regulators (Pandey et al. 2000).

19.4 Polyamines in Stress Responses

There is creditable evidence confirming the role of PAs in stress tolerance. Scavenging of excess reactive oxygen species (ROS) is a direct strategy by which plants adapt to adverse environments. However, altering metabolism and accumulating beneficial metabolites, including PAs, are another ways of plants' defense mechanism. Often changes in PA metabolism and expression levels of their pathway genes are positively linked with enhanced tolerance of abiotic stresses in plants (Shi and Chan 2014). For example, overexpression of *FcWRKY70* resulted in Put accumulation to provide drought tolerance in *Fortunella crassifolia* (Gong et al. 2015). Spd promotes biomass accumulation and upregulates proteins involved in cell rescue. Spd was involved in inducing antioxidant enzymes in tomato (*Lycopersicon esculentum*) seedlings subjected to high-temperature conditions (Sang et al. 2017). Exogenous Spm treatment induced defense mechanism and caused resistance against a root rot pathogen, *Phytophthora capsici,* in *Capsicum annuum* (Koc et al. 2017). Furthermore, Spd was found to be vital for adjustment of intracellular PA pathways and endogenous PA homeostasis, which enhanced salt tolerance in rice (Saha and Giri 2017).

19.5 Mode of Action of Polyamines in Stress Responses

PAs are reported to protect plants against stress through mechanisms including the following:

1. Polycationic PAs are known to bind anionic molecules such as nucleic acids and proteins, thus stabilizing them. This property may be important in preventing stress-induced damage to these macromolecules. Spd and Spm have been shown to prevent radiation and oxidative stress-induced strand breaks in DNA.
2. PAs are involved in the regulation of membrane transport in plants. They have been shown to block two slow and fast vacuolar cation channels. The effect of PAs is direct, and the channels open when PAs are withdrawn. PAs also affect vacuolar and plasma membrane H^+ and Ca^{2+} pumps and have been reported to bring about stomatal closure in response to drought stress by blocking the activity of a KAT1-like inward K^+ channel in the guard cell membrane. This is an indirect effect caused by low-affinity PA binding to the channel protein.
3. PAs are known to modulate ROS homeostasis. They are known for inhibiting metals auto-oxidation, thereby reducing the required electron supply for ROS generation. PAs are known to induce antioxidant enzymes in stress situations, as evidenced by use of inhibitors of PA biosynthesis or transgenics overexpressing enzymes involved in PA biosynthesis. For example, application of D-arginine,

which is an inhibitor of PA biosynthesis enzymes, resulted in reduced levels of PAs and increased levels of ROS. In addition, *ADC* overexpression influences increased tolerance to drought stress, causing reduced ROS generation in transgenic plants. PAs, especially Spm have roles as signaling molecules which activate the antioxidant machinery. Generation of H_2O_2 by PA catabolism, which may be promoted when PA levels are above a specific threshold, is known to play an essential role in signaling cascade regulation during abiotic and biotic stress conditions. Additionally, abiotic stresses often induce accumulation of abscisic acid (ABA) and nitric oxide (NO) and the interactions between them. PAs trigger protective responses, including regulation of the channels for ion homeostasis and stomatal responses to enhance and maintain water content, thereby inducing the antioxidant machinery to check excessive ROS generation, with compatible osmolytes synthesis and accumulation. All of these phenomena occur to cascade the abiotic stress tolerance of plants (Shi and Chan 2014).

19.6 Polyamines and Abiotic Stress

Polyamines modulate the plant's response to much broader range of abiotic stresses than expected, viz., drought, salinity, heavy metal toxicity, oxidative stress, chilling injury, high temperature, osmotic stress, water logging, and flooding tolerance as proved either by exogenous application of polyamines or by development of transgenic plants overexpressing the genes involved in polyamine biosynthesis (Gill and Tuteja 2010a, b). Increased or decreased levels of PAs either act as a signal or as a messenger (to transmit the perceived signals from the sensors) to articulate the plants' behavioral response spatially and temporally either to avoid or overcome stress. Modulated endogenous polyamine (free or conjugated or bound) levels are known to be involved in formation of polyamine-RNA complexes, thereby generating structural changes in RNA at physiological concentrations of potassium and magnesium ions (Igarashi and Kashiwagi 2000). Covalent linkage of polyamines to various enzymes or proteins (post-translational modification) involved in physiological processes under normal or stressed conditions was catalyzed by transglutaminase (TGase; EC 2.3.2.13) class of enzymes (Beninati et al. 1985; Folk 1980). Of various abiotic environmental stimuli under which polyamines get modulated and thereby its cellular functions were mineral nutrient deficiency (Richards and Coleman 1952; Coleman and Hegarty 1957), metal toxicity (Choudhary et al. 2012a, b), salinity (Lefèvre et al. 2001; Hummel et al. 2004), high (Oshima 2007) and low temperature (Hummel et al. 2004), drought (Bhatnagar et al. 2008; Alcazar et al. 2010), hypoxia (Moschou et al. 2008), osmotic (Lefèvre et al. 2001), and oxidative factors (Moschou et al. 2008; Bouchereau et al. 1999). PAs, also change ion channels (Takahashi and Kakehi 2010), stimulate special kind of protein synthesis, stimulate assembly of 30S ribosomal subunits, and stimulate Ile-tRNA formation (Igarashi and Kashiwagi 2000). Also, modulated titers of polyamines in combination with epibrassinolides, active form of brassinosteroids, were reported to regulate abscisic acid (ABA) and indole-3-acetic acid (IAA) pathways which in turn enhances tolerance to metal toxicity (Choudhary et al. 2012a, b). PAs in combination with brassinosteroids besides modulating ABA and IAA pathways with their cascading effects for heavy metal tolerance also modulate levels of antioxidants like glutathione, ascorbic acid, proline, glycine-betaine, and antioxidant enzymes like glutathione reductase, peroxidase, catalase, and superoxide dismutase to cause stress tolerance (Choudhary et al. 2012a, b). Enhanced levels of polyamines either through exogenous feeding (Gill and Tuteja 2010a, b) or through heterologous expression of polyamine biosynthetic genes in transgenic plants (Liu et al. 2007) were reported to increase abiotic stress tolerance. However, the use of constitutively expressed promoters like CaMV35S, ubiquitin, and actin with polyamine biosynthetic genes toward stress tolerance may produce modulated polyamine levels even under normal conditions resulting in deleterious effects which causes reduced plant yield special concern toward agricultural crops (Katiyar et al. 1999).

19.7 Polyamines Involvement in Regulation of Plant Stress Tolerance and Adaptation

Abiotic and biotic stresses cause alterations in the normal physiological processes of all plant organisms, including the economically important crops. Plant damage and productivity decrease are most often due to naturally occurring unfavorable factors of the environment—natural stress factors. Plant organisms are also imposed to a large scale with new stressors related to human activity—anthropogenic stress factors. Independent of the type of stress, an increased production of reactive oxygen species (ROS) occurs in plants that alter their normal physiological functions, decrease the biosynthetic capacity of plant organisms, and cause damage that may lead to plant death (Mittler 2002; Fujita et al. 2006; Ahmad et al. 2008; Gill and Tuteja 2010a, b; Potters et al. 2010). In plants, ROS are generated mainly as by-products of various processes requiring high metabolic activity or elevated electron flow by electron transport chains. The major targets of deleterious ROS action are cellular macromolecules as phospholipids, proteins, and nucleic acids. Plant organisms possess a complex of antioxidant protective systems in order to cope with destructive effects of the unfavorable environmental conditions. Beside enzymatic antioxidants and nonenzymatic antioxidants, a significant number of studies provided evidence that PAs also contribute to plant stress tolerance as a part of defense mechanisms or adaptation programs that help plants to mitigate the negative stress consequences. Since all stresses limit plant growth and crop productivity, the efforts of many scientists are focused to minimize the negative stress effects. A promising strategy to enhance plant tolerance and adaptation to an unfavorable environment is the use of a transgenic and molecular genetic approach to increase the cellular PA concentrations (Table 19.1).

Table 19.1 Enhanced stress tolerance in transgenic plants engineered to overproduce polyamines (PAs) Source: Todorova et al. (2014)

Gene	Gene source	Transgenic plant	Increased tolerance	PAs overproduction
ADC	Oat	Rice	Salt tolerance	Put
ADC	Oat	Eggplant	Multiple abiotic stress tolerance, fungus wilt tolerance	Put, Spd (particularly conjugated forms), and free Spm fraction
ADC	Datura	Rice	Drought tolerance	Put, facilitating synthesis of Spm and Spd
ADC1	*Arabidopsis*	*Arabidopsis*	Freezing tolerance	Put
ADC2	*Arabidopsis*	*Arabidopsis*	Drought and cold tolerance	Put
PtADC	*Trifoliate orange*	Tobacco tomato	Improvement in dehydration and drought tolerance	Put
PtADC	*Trifoliate orange*	*Arabidopsis* adc 1–1 mutant	High osmoticum, drought, low temperature tolerance	Put
ODC	Mouse	Tobacco	Salinity tolerance	Put
SAMDC	Tritordeum	Rice	Salt tolerance	Spm and Spd
SAMDC	Human	Tobacco	Salt, drought, fungal wilt tolerance	Spd, put especially conjugated fraction
SAMDC	Carnation	Tobacco	Salt, cold, acidic, ABA tolerance	Put, Spd, and Spm
ySAMDC	Yeast	Tomato	High temperature tolerance	Spm and Spd after exposure to high temperature
MdSAMDC2	Apple	Tobacco	Low temperature, salt, osmotic tolerance	Free put, Spd, and Spm
SAMDC1	*Arabidopsis*	*Arabidopsis*	Salt, dehydration, ozone tolerance	Spm
MdSAMDC1	Apple	European pear	Salt, osmotic tolerance	Spd
MdSAMDC1	Apple	European pear	Multiple abiotic stress (salt, osmotic, Cu) tolerance	Spd
MdSAMDC1	Apple	European pear	Al tolerance	Spd
MdSAMDC1	Apple	European pear	Heavy metal (Cd, Pb, Zn) tolerance	Spd
MdSAMDC1	Apple	Tomato	Salinity tolerance	Spd and Spm
SPDS	Figleaf gourd	*Arabidopsis*	Chilling, freezing, salinity, hyperosmosis, drought, paraquat tolerance	Conjugated Spd

(continued)

Table 19.1 (continued)

Gene	Gene source	Transgenic plant	Increased tolerance	PAs overproduction
SPDS (FSPD1)	Figleaf gourd	Sweet potato	Chilling heat	Spd
SAMS (SsSAMS2)	*Suaeda salsa*	Tobacco	Salt tolerance	Free Spm, Spd, and put

19.8 Abiotic Stress

19.8.1 Mineral Nutrient Deficiency

Since macro- and micronutrients are of paramount importance for normal plant growth and development, insufficient mineral nutrition affects all physiological processes in plants. To cope with mineral deficiency, plants usually augment PAs in response to nutrient shortage of potassium, boron, calcium, magnesium, and phosphorus (Wimalasekera et al. 2011). Boron deficiency caused an accumulation of free and conjugated Put and Spd (Camacho-Cristobal et al. 2005). Polyamines are also incremented by lower P-supply in roots and shoots of *Plantago lanceolata* L. (Bratek and Lang 2003). Plants grown on soils with low Ca concentration due to acidic deposition are exposed to multiple stress factors, and under these conditions, plants produce stress-related N-rich metabolites like Put, arginine, γ-aminobutyric acid, and proline in a species-particular manner for protection from toxic ammonia (Minocha et al. 2010). Potassium deficiency leads to increased levels of free PAs in *Betula pendula* and *Betula pubescens* (Sarjala and Kaunisto 2002) and cause a time-dependent pattern of Put accumulation during in vitro development of *Gentiana triflora* (Takahashi et al. 2012). Increased free-Put in potassium-deficient plants indicates that PAs are involved in maintenance of cation–anion balance in plant cells and conferred plant adaptation to ionic stress (Bouchereau et al. 1999).

19.8.2 Drought, Salt, Cold, and Osmotic Stress

Drought and salinity are widespread environmental constraints, and both cause reduced water potential and may lead to oxidative stress in plants (Lei 2008; Wang et al. 2011). The negative effects of salinity are deleterious involving complex mechanism, and they often come along ion toxicity. Drought increases accumulation of Na^+ in cells and also results in ion toxicity (Wang et al. 2003; Basu et al. 2010). An increase of PAs due to drought and osmotic stresses has been found in different plant tissues (Yang et al. 2007; Lei 2008; Sziderics et al. 2010; Ghosh et al. 2011). Moderate or severe drought increased lipid peroxidation but also proline and PAs in wheat seedlings (Todorova et al. 2008). Frequently, PA accumulation (primarily Spd and/or Spm) is associated with drought and salt tolerance, supporting the effect of

PAs in response to stress and tolerance mechanisms (Basu et al. 2010; Zhou and Yu 2010; Alet et al. 2011, 2012). Mutlu and Bozcuk (2007) noted that the concentrations of free, bound, and total Spm increased in roots of *Helianthus annuus* L. (salt-tolerant and salt-sensitive) treated with NaCl and assumed its role in diminishing the harsh consequences of salinity. Salt tolerance in sunflower plants was related to the excessive accumulation of PAs in roots which were grown under salt stress conditions. Accumulation of Spm and compatible solutes (sucrose, proline, mannitol, and raffinose) was shown to be main players in alleviating NaCl stress in *Populus tremula* (Jouve et al. 2004). The role of higher PAs in osmotic adjustment as compatible solutes has been suggested (Basu et al. 2010; Alcazar et al. 2011a; Hussain et al. 2011).

Recently, Gupta et al. (2012a) reported that NaCl and Spd spray in rice (salt-tolerant and salt- sensitive) led to phosphorylation of 42-kDa Ca^{+2}-independent SnRK2 in roots of rice because of Spd in response to NaCl. Gupta et al. (2012b) demonstrated the important role of Spd in regulation of salinity-mediated signaling in rice. The response of plant varieties differing in tolerance to drought or salinity includes Put accumulation for sensitive cultivars and increase of Spm and Spd for tolerant varieties of rice (Roy et al. 2005; Roychoudhury et al. 2008; Basu et al. 2010), wheat (Liu et al. 2004b), and barley (Liu et al. 2006). Zhou and Yu (2010) demonstrated that rise of free and conjugated Spd and Spm in vetiver grass leaves assist plants to cope with water deficit conditions. Conversely, withholding water for 1 week in the drought-sensitive crop pepper caused significant accumulation in Put and cadaverine in leaves of pepper (Sziderics et al. 2010). Legocka and Sobieszczuk-Nowicka (2012) documented reduced amounts of microsome- and thylakoid-associated PAs in *Zea mays* and *Phaseolus vulgaris* subjected to iso-osmotic concentrations of NaCl and sorbitol. The authors suggested that these cultivars are drought sensitive and noted that PAs associated with microsomes and thylakoids might be good markers of plant stress tolerance. In order to show the effect of ionic and osmotic components of salinity on free PAs, Lefèvre et al. (2001) studied short-term exposure of salt-resistant and salt-sensitive rice cultivars to PEG or iso-osmotic concentrations of NaCl and KCl. The authors demonstrated that both ion and PA concentrations were changed 3 h after ionic stresses and assumed that the ionic component may trigger short-term PA accumulation independent of the osmotic component. The authors reported that enhanced Put levels also depends on the plant tissue in rice treated with PEG, KCl, or NaCl; it was found in the roots of salt-resistant cultivar, whereas in shoots for salt-sensitive cultivar. Similarly, Hummel et al. (2004) showed that the major effect, even after long-term exposure of *Pringlea antiscorbutica* seedlings to salinity and osmotic stress, was the modification of PA distribution between roots and shoots. Higher PA content in roots was assumed to be a developmental response to stress, and their accumulation in roots facilitated reinitiation of root growth. In contrast, salinity resistance in rice exposed to short-term PEG, KCl, and NaCl was not related with Spm or Spd (Lefèvre et al. 2001). A relationship between stress-induced augmentation of Spd and Spm in transgenic plants and tolerance to drought stress was also demonstrated. In addition, Peremarti et al. (2009) generated transgenic rice plants overexpressing *DsSAMDC* in order to increase only higher PAs but not Put concentrations and noted accumulation of Spm, which facilitates drought recovery.

Alcazar et al. (2011b) analyzed *Arabidopsis* transcriptional profiles of PA biosynthetic genes and their metabolic fluxes through progressive drought acclimation with assessing the amounts of PAs in resurrection plant *Craterostigma plantagineum* and in PA biosynthetic mutants of *Arabidopsis*. The Put to Spm conversion in *Arabidopsis* coupled with Spm to Put back conversion has been proposed to participate in the drought stress response and to increase an effective PA recycling-loop during acclimation to water shortage. Involvement of Spm and Spd in maintaining ionic homeostasis by regulation of the activity of plasma membrane H^+-ATPase and vacuolar H^+-ATPase activities has been proposed as a general mechanism for salt tolerance and confirmed in different model systems (Roy et al. 2005; Liu et al. 2006; Janicka-Russak et al. 2010; Orsini et al. 2011). Similarly, due to experiments with PEG-treated drought-tolerant and drought-sensitive wheat cultivars, Liu et al. (2004a) reported that PAs conjugated to tonoplast vesicles correlate with maintenance of tonoplast H^+-ATPase and H^+-PPase activities in roots with enhanced osmotic stress tolerance in the plant. The authors could not detect Spm occurrence in wheat roots but free fractions of Spm- and Spd-enhanced osmotic stress tolerance in seedlings of the same cultivars (Liu et al. 2004b).

Yamaguchi et al. (2006, 2007) showed that the double knockout mutant of *Arabidopsis* (*acl5/spms*), which is unable to produce Spm, is hypersensitive to salinity and drought, but the addition of Spm specifically rescues the hypersensitive phenotype of mutant plants. The *acl5/spms* plant was shown to be Ca^{2+} deficient (Yamaguchi et al. 2006), and as a result of improper stomatal closure under drought conditions, it lost more water as compared to the control (Yamaguchi et al. 2007). The authors proposed a model for the defensive role of Spm in salinity and drought stress responses (Yamaguchi et al. 2006; Kusano et al. 2007).

The expression of certain genes is also induced by ABA treatment (Urano et al. 2003). The *ADC2*, *SPDS1*, and *SPMS* expression was examined in ABA-deficient (*aba2–3*) and ABA-insensitive (*abi1–1*) mutants exposed to water stress exhibiting decreased transcriptional induction in the stressed *aba2–3* and *abi1–1* mutants compared to the wild type, showing that ABA modulates polyamine metabolism at the transcription level by upregulating the expression of *ADC2*, *SPDS1*, and *SPMS* genes under water stress conditions (Alcázar et al. 2006a, b). In addition, Put accumulation, occurred as a result of drought, is also damaged in the *aba2–3* and *abi1–1* mutants in comparison with wild-type plants. All these observations show that upregulation of PA-biosynthetic genes and accumulation of Put under water stress are especially due to ABA-dependent responses. It is assumed that polyamine responses to salt stress are also ABA-dependent, since both *ADC2* and *SPMS* are produced by ABA. In fact, stress-responsive, drought-responsive (DRE), low temperature-responsive (LTR), and ABA-responsive elements (ABRE and/or ABRE related motifs) are there in the promoters of the polyamine biosynthetic genes (Alcázar et al. 2006). This strengthens the view that in response to drought and salt treatments, the expression of some of the genes in polyamine biosynthesis are controlled by ABA. Free Put levels are enhanced on cold treatment, and this corresponds with the introduction of *ADC* genes. Reduced expression of *NCED3* and several ABA-regulated genes was detected in the *adc1* mutants at low temperature. Complementation analyses of *adc1* mutants with ABA and mutual complementation of *aba2–3* mutant with Put supported the conclusion that diamine controls the levels of ABA in response to cold by modulating ABA biosynthesis at the transcriptional level (Cuevas et al. 2008, 2009). Certainly, Put and ABA are integrated in a positive feedback loop in which ABA and Put work synergistically for biosynthesis as a result of abiotic stress conditions that culminates a unique mode of operation of polyamines as regulators of ABA biosynthesis.

Liu et al. (2004a, b, c) reported that polyethylene glycol (PEG 6000) treatment (inducing water stress) significantly increased the free Spd and free Spm levels in the leaves of *Triticum aestivum* drought-tolerant cv. Yumai No. 18, whereas Yangmai No. 9 cv. (drought-sensitive) showed a significant increase of free Put. They suggested that free Spd, free Spm, and PIS-bound Put facilitated the osmotic stress tolerance of wheat seedlings. In response to PEG 6000 (20% w/v, 48 h)-mediated water stress condition in rice seedlings, Basu et al. (2010) showed that the salt-tolerant rice variety Pokkali accumulated the highest levels of Spd, Spm, and total PAs, while the Put level was the highest in the salt-sensitive variety IR-29 and induced maximally in the aromatic rice variety Pusa Basmati. In this respect, the aromatic variety behaved more closely to the sensitive variety. It was found that acquired tolerance to low water potential in potato cells leads to changes in Put biosynthesis and conjugation, which may be involved in ensuring cell survival (Gill and Tuteja 2010a, b). In case of *Theobroma cacao*, the expression of *TcODC*, *TcADC*, and *TcSAMDC* was induced with the onset of drought and correlated with the changes in stomatal conductance, photosynthesis, PSII efficiency, and leaf water potential. Induction of *TcSAMDC* in the leaves was more closely correlated with the changes in water potential (Bae et al. 2008). Yang et al. (2007) suggested that under drought conditions, the drought-resistant rice cultivars have the ability to respond early to water stress through increases in PA levels. Drought-resistant cultivars have higher SAMDC and SPDS activities and accumulated higher free Spd and free Spm in the leaves than drought-susceptible ones under water stress. The drought resistance in rice is therefore not only connected with PA levels but also with the response time at which PAs are significantly elevated. Vetiver grass can cope well with a moderate water-deficit conditions through maintenance of the total contents of free, conjugated, and bound Spd and Spm in leaves (Zhou and Yu 2010). In tolerant grapevine cultivars only, the higher PA-biosynthetic rate in the cellular compartment eliminates detrimental effects exerted by PAO-derived H_2O_2 in the apoplast. In the sensitive genotype, intracellular homeostasis of PAs is not restored, and their levels are insufficient to mitigate the intervening effects of H_2O_2 (Toumi et al. 2010). Endogenous levels of individual as well as total PAs in the roots of 7-day-old chickpea seedlings subjected to -0.8 MPa water stress increased significantly (Nayyar et al. 2005).

Using *Arabidopsis* has opened new vistas in functional dissection of the polyamine metabolic pathway and its role in the control of abiotic stress responses (Ferrando et al. 2004; Alcázar et al. 2006b; Kusano et al. 2008; Takahashi and

Kakehi 2010; Gill and Tuteja 2010a). Annotation of the *Arabidopsis* genome enabled complete compilation for the biosynthetic pathway of polyamine. Transcript prowling by using Q-RT-PCR has revealed that water stress induces the expression of *ADC2*, *SPDS1*, and *SPMS* genes (Alcázar et al. 2006a). The expression of some of these genes is also induced by ABA treatment (Perez-Amador et al. 2002; Urano et al. 2003). To get a further insight into ABA regulation of polyamine pathway, the expression of *SPMS, SPDS1*, and *ADC2* was investigated in the ABA-deficient (*aba2–3*) and ABA-insensitive (*abi1–1*) mutants subjected to water stress (Alcázar et al. 2006a). These three genes display reduced transcriptional induction in the stressed *aba2–3* and *abi1–1* mutants compared to the wild type, showing that ABA modulates polyamine metabolism at the transcription level by upregulating the expression of *ADC2*, *SPDS1*, and *SPMS* genes under water stress conditions (Alcázar et al. 2006a). In addition, Put accumulation in response to drought is also hampered in the *aba2–3* and *abi1–1* mutants compared to wild-type plants. This result is further supported by metabolomic studies showing that polyamine responses to dehydration are also impaired in *nced3* mutants (Urano et al. 2009). All these observations support the conclusion that upregulation of PA-biosynthetic genes and accumulation of Put under water stress are mainly ABA-dependent responses. Under salt stress conditions, there is a rapid increase in the expression of *ADC2* and *SPMS*, which is maintained during the 24-h treatment and results in increased Put and Spm levels (Urano et al. 2003). Spm-deficient mutants are salt sensitive, while the introduction of Spm suppresses the salt sensitivity, suggesting a protective role of Spm to high salinity (Yamaguchi et al. 2006).

It is evident that polyamine responses to salt stress are also ABA-dependent, since both *ADC2* and *SPMS* are induced by ABA (see above). In fact, stress-responsive, drought-responsive (DRE), low temperature-responsive (LTR), and ABA-responsive elements (ABRE and/or ABRE-related motifs) are available in the promoters of the polyamine biosynthetic genes (Alcázar et al. 2006b). This reinforces the view that in response to drought and salt treatments, the expression of some of the genes involved in polyamine biosynthesis are regulated by ABA. Transcript prowling has also revealed that cold enhances the expression of *ADC1*, *ADC2*, and *SAMDC2* genes (Urano et al. 2003; Cuevas et al. 2008, 2009). Free Put levels are enhanced on cold treatment, and this corresponds with the induction of *ADC* genes. Surprisingly, the levels of free Spd and Spm remain unchanged or even decrease in response to cold treatment. The absence of correlation between enhanced *SAMDC2* expression and the decrease of Spm levels could be a result of increased Spm catabolism (Cuevas et al. 2008). Since double mutants completely devoid of ADC activity are not viable in *Arabidopsis* (Urano et al. 2005), two independent mutant alleles for both *ADC1* and *ADC2* were used to study their response to freezing. As indicated in “polyamines and abiotic stress,” the *adc1* and *adc2* mutations caused higher sensitivity to freezing conditions, in both acclimated and non-acclimated plants, while addition of Put complemented this stress sensitivity (Cuevas et al. 2008, 2009). Reduced expression of *NCED3* and several ABA-regulated genes was found in the *adc1* mutants at low temperature. Complementation analyses of *adc1* mutants with ABA and reciprocal complementation of *aba2–3* mutant with Put supported the conclusion that this diamine controls the levels of ABA in response to cold by modulating ABA biosynthesis at the transcriptional level (Cuevas et al. 2008, 2009). All these results suggest that Put and ABA are integrated in a positive feedback loop, in which ABA and Put reciprocally promote each other's biosynthesis in response to abiotic stress. This highlights a novel mode of action of polyamines as regulators of ABA biosynthesis.

In general, cold-tolerant varieties show higher endogenous PA levels in response to low temperature than non-tolerant ones. Nayyar (2005) found that PA levels were enhanced by six to nine times in chickpea (*Cicer arietinum* L.) subjected to chilling temperatures. In poplar seedlings grown at 4 °C, Put accumulation occurred during the beginning of the cold treatment, while Spd and Spm accumulated after 4 or 7 days, respectively (Renaut et al. 2005). More recent quantitative expression analyses indicate that transcription of both *ADC1* and *ADC2* genes is produced as early as 30 min after cold exposure, the mRNA transcript levels of *ADC1* being higher than that of *ADC2*. The complementation analysis of *adc* mutants with ABA and reciprocal complementation tests of the *aba* 2–3 mutant with Put help the research finding of Put controls ABA levels in response to low temperature (Cuevas et al. 2008). However, data obtained by Kim et al. (2002) in tomato showed that ABA and Put affected cold-induced changes in cellular membranes of tomato leaves independently. The sequence analysis of the *ADC1* promoter revealed the presence of CRT/DRE, which could mediate the early and transient *ADC1* upregulation under cold stress (Alcázar et al. 2006b). Accumulation of Put was also observed in lucerne and wheat during cold hardening, in which a differential regulation of *ADC* activity could be detected between control and cold-treated plants. In a chilling-tolerant rice cultivar (Tainung 67), the *ADC* activity and Put levels increased during low temperature in both shoots and roots, while a chilling-sensitive cultivar (Taichung Native 1) showed a slight Put rise in shoots and a lowering in roots (Lee et al. 1997). Chilling induced the expression of all the three *ADC* genes (*MADC1, MADC2*, and *MADC3*) in *Brassica juncea*, whereas salt predominantly resulted in increased accumulation of *MADC3* transcript (Mo and Pua 2002). The low temperature also increased the Put level in cold-sensitive maize plants, especially when the stress occurred in light. Pillai and Akiyama (2004) suggested that the induction of the *OsSAMDC* gene in response to cold could be utilized as a molecular marker for the ability of rice seedlings to withstand low temperatures. In *Arabidopsis*, the expression of *SAMDC2* also increased after cold treatment. Cuevas et al. (2008) reported absence of correlation between enhanced *SAMDC2* expression and decrease of Spm levels in response to cold treatment, which may be a result of increased Spm catabolism. Shen et al. (2000) showed that chilling markedly increased Spd content concomitantly with a rise in SAMDC activity in the cold-tolerant cucumber cultivars (Jinchun No. 3) but not in sensitive ones (Suyo). This response is not mediated by ABA, since Spd pretreatment did not affect the content of ABA in cold-treated leaves of the cv. Suyo, and ABA content did not increase in the leaves of the cv. Jinchun No. 3. Imai et al. (2004) reported that a novel *SPDS* gene, *OsSPDS2* from rice, was involved in chilling response in roots. This gene is closely related to *AtSPDS3* or *At5g53120*, a putative *Arabidopsis SPDS* gene. Overexpression of *ZAT12*, a cold-inducible gene encoding a C2H2 zinc finger transcription factor (that contributes to an increased freezing tolerance), dampens the expression of the *CuAO* gene *At4G12280*, which shows sequence similarities to diamine oxidases. ZAT12 signaling promotes tolerance by activating a ZAT12 regulon, which includes *ADC1* and *ADC2* upregulation, leading to a resultant accumulation of Put (Alcázar et al. 2006b). Another cold inducible zinc finger protein called SCOF-1 has been reported from soybean (Kim et al. 2001).

Osmotic treatments using sorbitol induced high levels of Put and ADC in detached oat leaves (Flores and Galston 1984), whereas Spd and Spm show a dramatic decrease. Bouchereau et al. (1999) reported that osmotica with widely different assimilation routes, such as sorbitol, mannitol, proline, betaine, and sucrose, all induce a rise in Put. Such alterations are coincident with indications of stress, such as wilting and protein loss. Tiburcio et al. (1995) reported that when peeled oat leaves are incubated with sorbitol in the dark, they lose chlorophyll and senescence rapidly. Senescence could be slowed down by incorporating Spm in the incubation medium. The senescence-retarding effect of Spm was correlated with increase in the introduction of identified precursors into proteins, RNA, and DNA. They also inferred with rise in putrescine level and in particular its bound form to thylakoid membranes. A study by Schraudner et al. (1990) also discovered a correlation between ethylene emission, and PA biosynthesis was found in O^{3-} treated potato and tobacco plants, of which the leaves showed early senescence in response to the pollutant. In the presence of O_3, all compounds of ethylene biosynthetic pathway in tobacco leaves were upregulated. Put and Spd levels also increased, as did ornithine decarboxylase (ODC) activity (Bouchereau et al. 1999). Differences in PA (Put, Spd, Spm) response under salt stress have been reported among and within species. For example, according to Prakash and Prathapsenan (1988), endogenous levels of PAs (Put, Spd, and Spm) decreased in rice seedlings under NaCl stress, whereas Basu et al. (1988) found that salinity influences accumulation of such compounds in the same material (Bouchereau et al. 1999).

Santa-Cruz et al. (1997) reported that the (Spd + Spm):Put ratios increased with salinity in the salt-tolerant tomato species (*Lycopersicon pennellii*, Carrel D'Arcy) but not in the salt-sensitive tomato species (*L. esculentum*). In both species, stress treatments decreased the levels of Put and Spd. The Spm levels did not decrease with salinity in *L. pennellii* over the salinization period; however, it was found to decrease in *L. esculentum*. The effects of different NaCl concentrations on maize embryogenic cells obtained from immature embryo cultures of a salt-sensitive inbred line (cv. w64) and a resistant hybrid (cv, Arizona) have also been reported where increased salt concentration strongly reduced the growth of the calluses and resulted in a significant increase in the total PA (Put, Spd) content, especially caused by a rise in Put. A study conducted by Bouchereau et al. (1999) revealed that incorporating the inhibitors of Put synthesis, the ADC pathway in tomato plants functions appropriately in both stress and control conditions, whereas the ODC pathway is stimulated only under the stress conditions.

19.9 Heavy Metals

Metal contamination of soils has considerably increased due to human activities, and heavy metal excess also provoked changes in PA metabolism. Abundance of PAs in different plants cultivated on abundant heavy metals and their effect in regulation of plant tolerance and adaptation to metal stress is well studied. Cadmium excess led to increase in Put, which was accompanied by a respective decrease in higher PAs in *Potamogeton crispus* L. (Yang et al. 2010). The activities of PAO and DAO increased categorically with the rise in Cd concentrations, and authors suggested that some PAs and their forms impart an important role in the adaptation mechanism of *P. crispus* under Cd-stress.

In experiments with tobacco BY-2 cells exposed to Cd, a marked accumulation of total PAs during 3-day treatment was observed (Kuthanová et al. 2004). The increase in PA levels was because of Put which was coincident with DAO activity. Cd treatment increased PAs in roots and leaves of *Salix alba* L., whereas in *Populus nigra* L., only the Put content was significantly enhanced in roots (Zacchini et al. 2011). Lefèvre et al. (2009) also observed that the halophyte *Atriplex halimus* exposed to Cd accumulated free PAs. Probably the resistance of *A. halimus* to Cd toxicity was related to the improved tissue tolerance through increase in the synthesis of osmoprotective compounds like PAs. Polyamine concentrations in mung bean plants grown on medium supplied with Cd was also higher than these found in control plants and negatively correlated with the activity of DAO (Choudhary and Singh 2000).

Increased contents of Ni, Zn, Cu, and Cd in soil increased the accumulation of Put and Spm in spinach and barley (Bergmann et al. 2001). Groppa et al. (2007a, b, 2008) found that PA metabolism was differently affected in wheat and sunflower discs subjected to either Cu or Cd. Put content increased significantly in sunflower plants after Cd and Cu treatments, Spd was modified only by Cd, while Spm was affected by Cu or Cd and rose at 16 days after the germination of seeds (Groppa et al. 2007b). Both ADC and ODC activities were increased by Cd, whereas Cu enhanced ADC but reduced ODC activity. Furthermore, Groppa et al. (2007a) studied the PA metabolism in wheat leaves under Cd or Cu stress and showed that cadmium and copper increased Put, Spd remained unaltered either with Cd or Cu, while Spm was reduced as compared to control values by both heavy metal treatments. Copper also decreased ADC but did not modify ODC and DAO activities (Groppa et al. 2007a). Both metals caused significant increases in proline and Put and also in Spd and Spm at the highest concentration tested (1 mM), and these increments were more pronounced for Cd treatment of sunflower seedlings (Groppa et al. 2008). Additionally, the authors suggested that PAs are key biological compounds, probably involved in signaling pathways triggered under stress environmental conditions. Similarly, Zhang et al. (2010) found that Cu treatment increased Put and lowered the Spd and Spm levels, thereby reducing the Put/(Spd + Spm) ratio in leaves of grafted and non-grafted cucumber seedlings. Grafting markedly reversed these Cu-induced effects for all Spd, Spr, and Put and partly regained the Put/(Spd + Spm) ratio in leaves. These results suggest that grafting can enhance the tolerance of cucumber seedlings to Cu stress by increasing the activities of antioxidants and the levels of Spd and Spm, decreasing the Put/(Spd + Spm) ratio and the levels of ROS, promoting free proline and soluble protein synthesis in cucumber seedling leaves (Zhang et al. 2010).

In *Erica andevalensis* hydroponically grown with supplied increasing Cu concentration (1 to 500 μM) in the nutrient medium, the heavy metal led to a significant rise of PAs in shoots (Rossini Oliva et al. 2010). High levels of PAs in shoots helped the authors to conclude that PAs impart a defensive mechanism in plant cells at high metal concentration. Accordingly, Choudhary et al. (2009) showed that Cu treatment resulted in to a marked increase in PAs in *R. sativus* seedlings. Szafranska et al. (2011) examined the effect of Cu on the regeneration of *Daucus carota* L. androgenic embryos of var. Feria and 1014 breeding line and also on PAs, proline contents, lipid peroxidation, and Cu accumulation after 16 and 24 weeks and found that the higher tolerance of Feria to oxidative stress may result from increased content of proline and higher contents of the Put and Spd. The authors concluded that variations in PA levels depend not only on the concentrations of heavy metal but also on plant species and cultivars, and PAs point to better protection of such cultivars that possess higher constitutive PA levels. Zhao et al. (2008) investigated the phytotoxic effect of Ni on *Hydrocharis dubia* leaves and showed that Ni induced lipid peroxidation, destroyed the structure and functions of membranes, altered the balance of nutrient elements, and caused the toxicity of *H. dubia*. Ni stress significantly increased Put and lowered Spd and Spm levels, thus significantly reducing the ratio of free (Spd + Spm)/Put in leaves, which has been considered as a signal under stress. The changes of PAs content and physiological and biochemical responses in *H. dubia* leaves at high metal concentrations led the authors to conclude that PAs may be involved in the adaptation of plants to Ni-induced stress. Pb treatment of *Potamogeton crispus* L. significantly increased the free Put and changed dynamically other PAs levels.

The activities of PAO and ADC were initially decreased and then enhanced with the increase in the Pb concentration (Xu et al. 2011). Accumulation of PAs in shoot tissue of *Trifolium pratense* L. was affected by arsenic (Mascher et al. 2002) when metal was supplied in concentration 10 mg/kg soil. Extra accumulation of Spm seemed to be a part of a protective mechanism against the destruction of membranes caused by arsenate. In vitro shoots of a transgenic European pear, overexpressing *MdSPDS1*, performed attenuated susceptibility to heavy metal (Zn, Pb, Cu, Al, Cd) stress in conjunction with the wild plant (Wen et al. 2008, 2009, 2010). The authors supposed that abundance of Spd in transgenic plants could play an important role in the tolerance to heavy metals in long-term experiments. In this line Spd reduced the toxic effects of supplied metals mainly via alleviating oxidative status, by affecting mineral element balance and/or by its specific feature to act as metal chelator as well (Løvaas 1997; Lomozik et al. 2005). Thus, there is an indication that PAs acts as antioxidants and/or metal chelators (Kuthanová et al. 2004).

19.10 Herbicides

The extensive use of herbicides is a common practice in modern agriculture. However, their application usually provokes oxidative stress not only in sensitive but also in tolerant plants. Most of the herbicides are known to generate ROS by direct involvement in radical production or by inhibition of key biosynthetic pathways (Prasad and Rengel 1998). Enhancing free higher PAs in lotus prevented paraquat-induced superoxide production in vivo (Cuevas et al. 2004). However, because of the natural tolerance of *L. glaber* to the herbicide, it was not possible to correlate the content of free PAs with their hypothetical inhibitory role during oxidative stress. So, the authors tested for paraquat toxicity in tobacco plant and found that high concentrations of herbicide induced an increase of free PA levels in crude extracts and intercellular fluids, while low herbicide concentrations increased only the free PAs in the intercellular fluids. These results suggested that PA metabolism in the apoplast is involved in the physiological response to oxidative stress and subsequently in plant stress tolerance (Cuevas et al. 2004).

Deng (2005) found that chlorsulfuron and glyphosate directly regulated the levels of arginine and ornithine in *Datura stramonium* L. seedlings, which were increased two to three times. Recently, Kielak et al. (2011) investigated the effect of glyphosate-based herbicide (Roundup Ultra 360 SL) and showed significant positive correlation between Roundup dose and over-accumulation of Put and Spd as well as total PA in model plant duckweed. Putrescine was synthesized first within the plant cells and Spd later. Additionally, with augmentation of PAs, an increase in APX and CAT activities was recorded by herbicide which is the resultant of PA inclusion. These findings indicated that duckweed plants responded to herbicide stress by activating one or more components of the plant defense system, including PAs, which helps plants to mitigate the negative stress outcome.

19.11 Heat Tolerance

The amylose levels in rice are greatly decreased during elevated temperature conditions at the time of seed germination, changing the fine amylopectin structure and producing more chalky grains (Asaoka et al. 2010; Inouchi et al. 2000) which hampers the rice yield and seed quality (Krishnan and Ramakrishnan 2011; Peter 2011). The occurrence of chalky grains of rice is typically caused by the unusual expressions of genes encoding starch synthase enzymes (Nishi et al. 2001; Tanaka et al. 2010). As PAs are known to be involved in grain filling and they might contribute to build up heat resistance of some cereals, the hybrid rice “YLY 689” was taken in an experiment to find the effects of exogenous spermidine (Spd) on seed quality under high temperature during the grain filling stage (Fu et al. 2019). The concentration of Spm and Spd in superior grains of rice was remarkably higher than that of the inferior grains (Cao et al. 2016). In this investigation, just after pollination, spikes were treated with Spd or cyclohexylamine (CHA). Interestingly, CHA is its synthesis inhibitor.

Recently, Fu et al. (2019) revealed that when the rice plants were transferred to 40 °C for 5-day heat treatment, it resulted into significant enhancement in the germination index, percentage, germination index, seedling shoot height, vigor index, and dry weight of seeds in case of Spd pretreatment, harvested at 35 days after pollination. In contrast, the CHA significantly decreased the seed germination and seedling growth. Also, Spd categorically enhanced the peroxidase (POD) activity and reduced the malondialdehyde (MDA) content in seeds. Nevertheless, after spraying with Spd, the endogenous content of spermidine and spermine and the expression of their synthetic genes, *spermidine synthase* (*SPDSYN)*, and *spermine synthase* (*SPMS1* and *SPMS2*) remarkably enhanced, although, the accumulation of amylose, total starch, and the expression of their related synthase genes, *soluble starch synthase II-3* (*SS II-3*) and *granules bound starch synthase I* (*GBSSI*), also rose to some magnitude indicating that exogenous Spd pretreatment could diminish he high temperature stress on rice seed grain filling and improve the rice seed quality, which could possibly be induced by upregulating endogenous polyamines and starch metabolism (Fu et al. 2019). Recent report also suggests that Spd is involved in heat tolerance in higher plants, which provides membrane stability and increase the reactive oxygen species (ROS) scavenging system. Cao et al. (2016) found that the content of Spd and Spm in superior grains of rice was significantly higher than that in inferior grains (Cao et al. 2016). The content of Spm and Spd in grains works in synergy with grain plumpness during the grain filling stage.

19.12 Plant Senescence

The PA metabolic enzymes activities and PAs contents change throughout the stages of plant growth. In whole plants, endogenous PAs and PA synthetase activity were recorded to be highest in the meristem and growing cells and lowest in senescent tissues. As leaves senescence, the chlorophyll content slowly reduces and so does ADC and ODC activities, while PAO and hydrolases activities increase rapidly. All of these changes can be blocked by the inclusion of exogenous PAs (Duan 2000; Cai 2009). A reduction in PA levels seems to be a significant prelude to senescence signals, or it may be that a decrease in PAs content is the senescence signal (Duan et al. 2006). Exogenous Spd and Spm treatments can increase the content of PAs in cut flowers and prolong their senescence and improve quality (Yang and He 2001; Cao 2010). In *Anthurium andraeanum*, the introduction of GA_3 + Spm by spraying slowed down the senescence of cut flowers stored at 20 °C and augmented the quality of the inflorescences (Simões et al. 2018). Delayed leaf senescence was recorded to be linked with a rise in Spm concentration, reduced ROS production, and increased NO levels (Sobieszczuk-Nowicka 2017). Polyamines appeared to slow down the process of senescence by suppressing the ethylene biosynthesis (Woo et al. 2013; Anwar et al. 2015). 0.1 mM Spd application on gerbera flowers or gerbera vase flower fortification with 10 mM Spd resulted in slowing down the senescence, while those sprayed with 1 mM Spd, 10 mM Spd, 0.1 mM Spm, 1 mM Spm, or mixed solution of 0.1 mM each of Put, Spd, and Spm showed accelerated senescence, with brown spots and yellowing of the petal rims starting from day 2 of treatment (Bagni and Tassoni 2006). Legocka and Sobieszczuk-Nowicka (2012) found that chlorophyll rapidly degraded, and Put accumulation inhibited protein degradation and reduced chlorophyll losses (Serafini-Fracassini et al. 2010; Cai et al. 2015). In peony, a PA synthesis inhibitor (0.1 Mm) extended the lifespan and delayed the cut flowers senescence, while PAs lessened the flower lifespan and accelerated flower senescence (Han 2016).

19.13 Conclusion and Future Prospects

Many scientists have reported different plants defence mechanisms exhibited during its growth progression. However, the practical mode of action and regulations of many aspects still remain uncovered. The biosynthesis of PAs is present in every cell and tissue of plants; however, the number of enzymes involved in these processes is limited. That is why PA biosynthesis proves to be is an experimental model for studying how they act in protecting plants against various biotic and abiotic stresses. From several decades, PAs are being employed using mutants, inhibitors, plant tissue culture, exogenous application, genetic transformation techniques, etc. Various studies have been reported in order to illustrate the role of PAs such as Spm and Spd which have increased levels when plants get exposed to stress conditions enzymes, indicating the homeostatic equilibrium that is maintained inside the plant cells incorporating enzymes.

PAs' role in combating abiotic stress could easily be inferred by studies performed on *Arabidopsis*, wherein PAs were found to act antagonistically against salinity and cold stress conditions, thereby providing tolerance against such factors (Roy et al. 2005; Liu et al. 2006; Janicka-Russak et al. 2010; Orsini et al. 2011). Similarly, exogenous spray of Spd and Spm helped in delayed plant senescence in cut flowers and vase flowers (Yang and He 2001; Cao 2010). PA levels were found to be enhanced in plants experiencing mineral deficiencies such as potassium, boron, calcium, magnesium, and phosphorus (Wimalasekera et al. 2011). Likewise, there are reports from various researchers of how PAs play a pivotal role in plant stress conditions.

However, a lot had been studied and reported about PAs, but there still exists a lacuna of understanding the enzymes involved during biosynthesis of PAs, localization of PAs at cellular and subcellular levels, translocation and movements of free PAs, and their interaction with other plant hormones. Therefore, more research needs to be done under these niche areas so as to have a better understanding of polyamines as a whole.

References

Ahmad P, Sarwat M, Sharma S (2008) Reactive oxygen species, antioxidants and signaling in plants. J Plant Biol 51:167–173

Alcazar R, Altabella T, Marco F et al (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. Springer, New York

Alcázar R et al (2006a) Abscisic acid modulates polyamine metabolism under water stress in *Arabidopsis thaliana*. Physiol Plant 128:448–455

Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T (2006b) Involvement of polyamines in plant response to abiotic stress. Biotechnol Lett 28: 1867–1876

Alcazar R, Cuevas JC, Planas J, Zarza X, Bortolotti C, Carrasco P, Salinas J, Tiburcio AF, Altabella T (2011a) Integration of polyamines in the cold acclimation response. Plant Sci 180:31–38

Alcazar R, Bitrian M, Bartels D, Koncz C, Altabella T, Tiburcio AF (2011b) Polyamine metabolic canalization in response to drought stress in *Arabidopsis* and the resurrection plant *Craterostigma plantagineum*. Plant Signal Behav 6(2):243–250

Alcázar R et al (2006) Abscisic acid modulates polyamine metabolism under water stress in *Arabidopsis thaliana*. Physiol Plant 128:448–455

Alet A, Sánchez D, Ferrando A, Fernandez-Tiburcio A, Alcazar R, Cuevas JC, Altabella T, Pico FM, Carrasco P, Menéndez AB, Ruiz OA (2011) Homeostatic control of polyamine levels under long-term salt stress in *Arabidopsis*. Changes in putrescine content do not alleviate ionic toxicity. Plant Signal Behav 6:1–6

Alet AI, Sánchez DH, Cuevas JC, Marina M, Carrasco P, Altabella T, Tiburcio AF, Ruiz OA (2012) New insights into the role of spermine in *Arabidopsis thaliana* under long-term salt stress. Plant Sci 182:94–100

Anwar R, Mattoo AK, Handa AK (2015) Polyamine interactions with plant hormones: crosstalk at several levels. Int J Hydrogen Energy 38:1039–1051

Asaoka M et al (2010) Effect of environmental temperature during development of rice plants on some properties of endosperm starch. Starch-Starke 36:189–193

Bae H, Kim SH, Kim MS, Sicher RC, Lary D, Stream MD, Natarajan S, Bailey BA (2008) The drought response of *Theobroma cacao* (cacao) and the regulation of genes involved in polyamine biosynthesis by drought and other stresses. Plant Physiol Biochem 46:174–188

Bagni N, Tassoni A (2006) The role of polyamines in relation to flower senescence. Floricult Ornament Plant Biotechnol 1536:855–856

Bais HP, Ravishankar GA (2002) Role of polyamines in the ontogeny of plants and their biotechnological applications. Plant Cell Tiss Org Cult 69:1–34

Basu HS, Schwietert HCA, Feuerstein BG, Marton LJ (1990) Effect of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. Biochem J 269:329–334

Basu R, Maitra N, Ghosh B (1988) Salinity results in polyamine accumulation in early rice (*Oryza sativa* L.) seedlings. Aust J Plant Physiol 15:777–786

Basu S, Roychoudhury A, Saha PP, Sengupta DN (2010) Comparative analysis of some biochemical responses of three indica rice varieties during polyethylene glycol-mediated water stress exhibits distinct varietal differences. Acta Physiol Plant 32:551–563

Beigbeder AR (1995) Influence of polyamine inhibitors on light independent and light dependent chlorophyll biosynthesis and on the photosyntetic rate. J Photochem Photobiol 28:235–242

Beninati S, Piacentini M, Argento-Ceru MP, Russo-Caia S, Autuori F (1985) Presence of di- and polyamines covalently bound to protein in rat liver. BBA-Gen Sub 841(1):120–126

Bergmann H, Machelett B, Lippmann B, Friedrich Y (2001) Influence of heavy metals on the accumulation of trimethylglycine, putrescine and spermine in food plants. Amino Acids 20: 325–329

Bhatnagar P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. Plant Cell Rep 27(3):411–424

Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999) Polyamines and environmental challenges: recent development. Plant Sci 140:103–125

Cai G, Sobieszczuknowicka E, Aloisi I, Al E (2015) Polyamines are common players in different facets of plant programmed cell death. Amino Acids 47:27–44

Cai Q (2009) Progress in physiology of plant polyamines. Fujian Sci Technol Rice Wheat 27:37–40

Camacho-Cristobal JJ, Maldonado JM, Gonzales-Fontes A (2005) Boron deficiency increases putrescine levels in tobacco plants. J Plant Physiol 162:921–928

Cao D (2010) Effects of polyamines on seed quality and germination of super sweet corn seeds during development. Zhejiang University, Hangzhou

Cao YY et al (2016) Growth characteristics and endosperm structure of superior and inferior spikelets of indica rice under high-temperature stress. Biol Plant 60:532–542

Choudhary A, Singh R (2000) Cadmium-induced changes in diamine oxidase activity and polyamine levels in *Vigna radiata* wilczek seedlings. J Plant Physiol 156:704–710

Choudhary S, Bhardway R, Gupta B, Dutt P, Kanwar M, Arora P (2009) Epibrassinolide regulated synthesis of polyamines and auxins in *Raphanus sativus* L. seedlings under cu metal stress. Braz J Plant Physiol 21(1):25–32

Choudhary SP, Kanwar M, Bhardwaj R, Yu JQ, Tran LP (2012b) Chromium stress mitigation by polyamine brassinosteroid application involves phytohormonal and physiological strategies in *Raphanus sativus* L. PLoS One 7(3):e33210

Choudhary SP, Oral HV, Bhardwaj R, Yu J, Tran LP (2012a) Interaction of brassinosteroids and polyamines enhances copper stress tolerance in *Raphanus sativus*. Oxford University Press, Oxford

Coleman RG, Hegarty MP (1957) Metabolism of dl-ornithine-2-14C in normal and potassium-deficient barley. Nature 179:376

Cona A, Rea G, Angelini R, Federico R, Tavladoraki P (2006) Functions of amine oxidases in plant development and defence. Trends Plant Sci 11:80–88

Cuevas J, Lopez-Cobollo R, Alcazar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio A, Ferrando A (2009) Putrescine as a signal to modulate the indispensable ABA increase under cold stress. Plant Signal Behav 4:219–220

Cuevas JC, Lopez-Cobollo R, Alcazar R, Zarza X, Koncz C, Altabella T, Salinas J, Tiburcio AF, Ferrando A (2008) Putrescine is involved in *Arabidopsis* freezing tolerance and cold acclimation by regulating abscisic acid levels in response to low temperature. Plant Physiol 148:1094–1105

Cuevas JC, Sánchez DH, Marina M, Ruiz OA (2004) Do polyamines modulate the *Lotus glaber* NADPH oxidation activity induced by the herbicide methyl viologen? Funct Plant Biol 31:921–928

D'Oraci D, Bagni N (1987) In vitro interactions between polyamines and pectic substances. Biochem Biophys Res Commun 148:1159–1163

Deng F (2005) Effects of glyphosate, chlorsulfuron, and methyl jasmonate on growth and alkaloid biosynthesis of jimsonweed (*Datura stramonium* L.). Pest Biochem Physiol 82:16–26

Duan G (2000) Effect of spermidine on protein contents and protease during senescence of excised wheat leaves. J Sichuan Teach Coll 21:44–47

Duan G, Huang Z, Lin H (2006) The role of polyamines in the ontogeny of higher plants. Acta Agric Boreali Occidentalis Sinica 15:190–194

Duan JJ, Guo SR, Kang YY, Jiao YS (2007) Effects of exogenous spermidine on polyamine content and antioxidant system in roots of cucumber under salinity stress. J Ecol Rural Environ 4(4): 11–17

El Ghachtoul N, Martin-Tangu J, Paynot M, Gianinazz S (1996) First report of the inhibition of arbuscular mycorrhizal infection of *Pisum sativum* by specific and irreversible inhibition of polyamine biosynthesis or by gibberellic treatment. FEBS Lett 385:189–192

Evans PT, Malmberg RL (1989) Do polyamines have roles in plant development? Annu Rev Plant Physiol Plant Mol Biol 40:235–269

Ferrando A, Carrasco P, Cuevas JC, Altabella T, Tiburcio AF (2004) Integrated molecular analysis of the polyamine pathway in abiotic stress signaling. In: Amancio S, Stulen I (eds) Nitrogen acquisition and assimilation in higher plants. Kluwer Academic Publishers, London, pp 207–230

Flores HE, Galston AW (1984) Osmotic stress-induced polyamine accumulation in cereal leaves: I. physiological parameters of the response. Plant Physiol 75:102–109

Folk JE (1980) Transglutaminases. Annu Rev Biochem 49:517–531

Fu Y et al (2019) Spermidine enhances heat tolerance of rice seeds by modulating endogenous starch and polyamine metabolism. Molecules 24:1395

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442

Galston AW, Kaur-Sawhney R (1995) Polyamines as endogenous growth regulators. In: Davies PJ (ed) Plant hormones: physiology, biochemistry and molecular biology, 2nd edn. Kluwer Academic Publishers, Dordrecht, pp 158–178

Galston AW, Kaur-Sawhney RK (1990) Polyamines in plant physiology. Plant Physiol 94:406–410

Ghosh N, Adak MK, Ghosh PD, Gupta S, Sen Gupta DN, Mandal C (2011) Differential responses of two rice varieties to salt stress. Plant Biotechnol Rep 5:89–103

Gill SS, Tuteja N (2010a) Polyamines and abiotic stress tolerance in plants. Plant Signal Behav 5: 26–33

Gill SS, Tuteja N (2010b) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Gong XQ, Zhang JY, Hu JB, Wang W, Wu H, Zhang QH, Liu JH (2015) FcWRKY70, a WRKY protein of *Fortunella crassifolia*, functions in drought tolerance and modulates putrescine synthesis by regulating arginine decarboxylase gene. Plant Cell Environ 38:2248–2262

Groppa MD, Ianuzzo MP, Tomaro ML, Benavides MP (2007b) Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. Amino Acids 32:265–275

Groppa MD, Tomaro ML, Benavides MP (2007a) Polyamines and heavy metal stress: the antioxidant behavior of spermine in cadmium- and copper-treated wheat leaves. Biometals 20:185–195

Groppa MD, Zawoznik MS, Tomaro ML, Benavides MP (2008) Inhibition of root growth and polyamine metabolism in sunflower (*Helianthus annuus*) seedlings under cadmium and copper stress. Biol Trace Elem Res 126:246–256

Gupta B, Gupta K, Sengupta DN (2012a) Spermidine-mediated in vitro phosphorylation of transcriptional regulator OSBZ8 by SNF1-type serine/threonine protein kinase SAPK4 homolog in indica rice. Acta Physiol Plant 34:1321

Gupta K, Gupta B, Ghosh B, Sengupta DN (2012b) Spermidine and abscisic acid-mediated phosphorylation of a cytoplasmic protein from rice root in response to salinity stress. Acta Physiol Plant 34:29–40

Han L (2016) Studies on mechanism of low temperature storage and polyamine impact in cut flowers of herbaceous peony postharvest senescence. Shandong Agricultural University, Shandong

Heby O, Persson L (1990) Molecular genetics of polyamine synthesis in eukaryotic cells. Trends Biochem Sci 15:153–158

Hummel I, Gouesbet G, El Amrani A, Aïnouche A, Couée I (2004) Characterization of the two arginine decarboxylase (polyamine biosynthesis) paralogues of the endemic subantarctic cruciferous species *Pringlea antiscorbutica* and analysis of their differential expression during development and response to environmental stress. Gene 342:199–209

Hussain SS, Ali M, Ahmad M, Siddique KHM (2011) Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. Biotechnol Adv 29:300–311

Igarashi K, Kashiwagi K (2000) Polyamines: mysterious modulators of cellular functions. Biochem Biophys Res Commun 271(3):559–564

Imai R, Ali A, Pramanik HR, Nakaminami K, Sentoku N, Kato H (2004) A distinctive class of spermidine synthase is involved in chilling response in rice. J Plant Physiol 161:883–886

Inouchi N et al (2000) The effect of environmental temperature on distribution of unit chains of rice amylopectin. Starch-Starke 52:8–12

Janicka-Russak M, KabaŁa K, Mlodzińska E, Klobus G (2010) The role of polyamines in the regulation of the plasma membrane and the tonoplast proton pumps under salt stress. J Plant Physiol 167:261–269

Jiuju D, Shirong G (2005) Effects of exogenous spermidine on salt tolerance of cucumber seedlings under NaCl stress. China Veg 12:8–10

Jouve L, Hoffmann L, Hausman J-F (2004) Polyamine, carbohydrate, and proline content changes during salt stress exposure of aspen (*Populus tremula* L.): involvement of oxidation and osmoregulation metabolism. Plant Biol 6:74–80

Kakkar R, Rai V, Nagar P (1998) Polyamine uptake and translocation in plants. Biología Plantarum 40(4):481–491

Katiyar SA, Agarwal M, Grover A (1999) Emerging trends in agricultural biotechnology research: use of abiotic stress-induced promoter to drive expression of a stress resistance gene in the transgenic system leads to high level stress. Curr Sci 77(12):1577

Kielak E, Sempruch C, Mioduszewska H, Klocek J, Leszczynski B (2011) Phytotoxicity of roundup ultra 360 SL in aquatic ecosystems: biochemical evaluation with duckweed (*Lemna minor* L.) as a model plant. Pestic Biochem Physiol 99:237–243

Kim JC, Lee SH, Cheong YH, Yoo CM, Lee SI, Chun HJ, Yun DJ, Hong JC, Lee SY, Lim CO, Cho MJ (2001) A novel cold-inducible zinc finger protein from soybean, SCOF-1, enhances cold tolerance in transgenic plants. Plant J 25:247–259

Kim TE, Kim SK, Han TJ, Lee JS, Chang SC (2002) ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*). Physiol Plant 115:370–376

Koc E, Islek C, Kasko Arici Y (2017) Spermine and its interaction with proline induce resistance to the root rot pathogen *Phytophthora capsici* in pepper (*Capsicum annuum*). Hortic Environ Biotechnol 58:254–267

Krishnan P, Ramakrishnan BJ (2011) Chapter three-high-temperature effects on rice growth, yield, and grain quality. Adv Agron 111:87–206

Kusano T, Berberich T, Tateda C, Takahashi Y (2008) Polyamines: essential factors for growth and survival. Planta 228:367–381

Kusano T, Yamaguchi K, Berberich T, Takahashi Y (2007) The polyamine spermine rescues *Arabidopsis* from salinity and drought stresses. Plant Signal Behav 2:251–252

Kuthanová A, Gemperlová L, Zelenková S, Eder J, Machá˘cková I, Opatrny Z, Cvikrová M (2004) Cytological changes and alterations in polyamine contents induced by cadmium in tobacco BY-2 cells. Plant Physiol Biochem 42(2):149–156

Lee T, Liu JJ, Pua EC (1997) Molecular cloning of two cDNAs (accession Nos. X95729 and U80916) encoding *S*-adenosyl-L methionine decarboxylase in mustard (*Brassica juncea* [L.] Czern & Coss). Plant Physiol 115:1287

Lefèvre I, Gratia E, Lutts S (2001) Discrimination between the ionic and osmotic components of salt stress in relation to free polyamine level in rice (*Oryza sativa*). Plant Sci 16:943–952

Lefevre I, Marchal G, Meerts P, Correal E, Lutts S (2009) Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L. Environ Exp Bot 65: 142–152

Legocka J, Sobieszczuk-Nowicka E (2012) Sorbitol and NaCl stresses affect free, microsome associated and thylakoid-associated polyamine content in *Zea mays* and *Phaseolus vulgaris*. Acta Physiol Plant 34:1145–1151

Lei Y (2008) Physiological responses of *Populus przewalskii* to oxidative burst caused by drought stress. Russ J Plant Physiol 55:857–864

Liu H, Liu Y, Yu B, Liu Z, Zhang W (2004a) Increased polyamines conjugated to tonoplast vesicles correlate with maintenance of the H+-ATPase and H+-PPase activities and enhanced osmotic stress tolerance in wheat. J Plant Growth Regul 23:156–165

Liu HP, Dong BH, Zhang YY, Liu ZP, Liu YL (2004b) Relationship between osmotic stress and the levels of free, conjugated and bound polyamines in leaves of wheat seedlings. Plant Sci 166: 1261–1267

Liu HP, Dong BH, Zhang YY, Liu ZP, Liu YL (2004c) Relationship between osmotic stress and the levels of free, conjugated, and alterations in polyamine contents induced by cadmium in tobacco BY-2 cells. Plant Physiol Biochem 42(2):149–156

Liu J, Yu B-J, Liu Y-L (2006a) Effects of spermidine and spermine levels on salt tolerance associated with tonoplast H + -ATPase and H + -PPase activities in barley roots. Plant Growth Regul 49:119–126

Liu JH, Kitashiba H, Wang J, Ban Y, Moriguchi T (2007) Polyamines and their ability to provide environmental stress tolerance to plants. Plant Biotechnol 24:117–126

Liu JH, Nada K, Honda C, Kitashiba H, Wen XP, Pang XM, Moriguchi T (2006) Polyamine biosynthesis of apple callus under salt stress: importance of the arginine decarboxylase pathway in stress response. J Exp Bot 57:2589–2599

Lomozik L, Gasowska A, Bregier-Jarzebowska R, Jastrzab R (2005) Coordination chemistry of polyamines and their interactions in ternary systems including metal ions, nucleosides and nucleotides. Coord Chem Rev 249:2335–2350

Lovaas E (1997) Antioxidant and metal-chelating effects of polyamines. In: Sies H (ed) Advances in pharmacology, Vol. 38: antioxidants in disease mechanisms and therapy. Academic Press, Amsterdam, pp 119–149

Malmberg RL, Watson MB, Galloway GL, Yu W (1998) Molecular genetic analysis of plant polyamines. Crit Rev Plant Sci 17:5

Martin-Tanguy J (1987) Hydroxycinnamic acid amides, hypersensitivity, flowering and sexual organogenesis in plants. In: Von Wettstein D, Chua DN (eds) Plant molecular biology. Plenum Publishing Corporation, New York, pp 253–263

Martin-Tanguy J (2001) Metabolism and function of polyamines in plants: recent development (new approaches). Plant Growth 34:135

Mascher R, Lippmann B, Holzinger S, Bergmann H (2002) Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. Plant Sci 163:961–969

Mehta HS, Saftner RA, Mehta RA, Davies PJ (1994) Identification of post transcriptionally modified 18-kilodalton protein from rice as eukaryotic translocation initiation factor 5A. Plant Physiol 106:1413–1419

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49: 69–76

Minocha R, Long S, Thangavel P, Minocha SC, Eagar C, Driscoll CT (2010) Elevation dependent sensitivity of northern hardwoods to Ca addition at Hubbard Brook Experimental Forest, NH, USA. For Ecol Manage 260:2115–2124

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mo H, Pua EC (2002) Up-regulation of arginine decarboxylase gene expression and accumulation of polyamines in mustard (*Brassica juncea*) in response to stress. Physiol Plant 114:439–449

Moschou PN, Paschalidis KA, Delis ID et al (2008) Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H2O2 signatures that direct tolerance responses in tobacco. Plant Cell 20(6):1708–1724

Mutlu F, Bozcuk S (2007) Salinity-induced changes of free and bound polyamine levels in sunflower (*Helianthus annuus* L.) roots differing in salt tolerance. Pak J Bot 39:1097–1102

Nayyar H (2005) Putrescine increases floral retention, pod set and seed yield in cold stressed chickpea. J Agron Crop Sci 191:340–345

Nayyar H, Kaur S, Singh S, Kumar S, Singh KJ, Dhir KK (2005) Involvement of polyamines in the contrasting sensitivity of chickpea (*Cicer arietinum* L.) and soybean (*Glycine max* (L.) Merrill.) to water deficit stress. Bot Bull Acad Sin 46:333–338

Nishi A et al (2001) Biochemical and genetic analysis of the effects of amylose-extender mutation in rice endosperm. J Plant Physiol 127:459

Orsini F, Accorsi M, Gianquinto G, Dinelli G, Antognoni F, Ruiz Carrasco KB, Martinez EA, Alnayef M, Marotti I, Bosi S, Biondi S (2011) Beyond the ionic and osmotic response to salinity in *Chenopodium quinoa*: functional elements of successful halophytism. Funct Plant Biol 38(10):818–831

Oshima T (2007) Unique polyamines produced by an extreme thermophile, *Thermus thermophilus*. Amino Acids 33:2

Pandey S, Ranade S, Nagar P, Nikhil K (2000) Role of polyamines and ethylene as modulators of plant senescence. J Biosci 25(3):291–299

Paradi I, Bratek Z, Lang F (2003) Influence of arbuscular miccorhiza and phosphorus supply on polyamine content, growth and photosynthesis of *Plantago lanceolata*. Biologia Plantarum 46(4):563–569

Peremarti A, Bassie L, Christou P, Capell T (2009) Spermine facilitates recovery from drought but does not confer drought tolerance in transgenic rice plants expressing *Datura stramonium* S-adenozylmethionine decarboxylase. Plant Mol Biol 70:253–264

Perez-Amador MA, Leon J, Green PJ, Carbonel lJ. (2002) Induction of the arginine decarboxylase ADC2 gene provides evidence for the involvement of polyamines in the wound response in *Arabidopsis*. Plant Physiol 130:1454–1463

Peter GJ (2011) Regulation of starch biosynthesis in response to a fluctuating environment. Plant Physiol 155:1566–1577

Pillai MA, Akiyama T (2004) Differential expression of an S-adenosyl-L-methionine decarboxylase gene involved in polyamine biosynthesis under low temperature stress in japonica and indica rice genotypes. Mol Genet Genomics 271:141–149

Pohjanpelto P, Höltta E (1996) Phosphorylation of Okazaki-like DNA fragments in mammalian cells and role of polyamines in the processing of this DNA. EMBO J 15:1193–1200

Potters G, Horemans N, Jansen MAK (2010) The cellular redox state in plant stress biology – a charging concept. Plant Physiol Biochem 48:292–300

Prakash L, Prathapasenan G (1988) Effect of NaCl salinity and putrescine on shoot growth, tissue ion concentration and yield of rice (*Oryza sativa* L. GR3). J. Agric Crop Sci 160:325–334

Prasad M, Rengel Z (1998) Plant Acclimation and adaptation to natural and anthropogenic stress. Ann N Y Acad Sci 851(1):216–223

Renaut J, Hoffmann L, Hausman JF (2005) Biochemical and physiological mechanisms related to cold acclimation and enhanced freezing tolerance in poplar plantlets. Physiol Plant 125:82–94

Richards FJ, Coleman RG (1952) Occurrence of putrescine in potassium-deficient barley. Nature 170:460

Rossini Oliva S, Mingorance MD, Valdes B, Leidi EO (2010) Uptake, localisation and physiological changes in response to copper excess in *Erica andevalensis*. Plant and Soil 328:411–420

Roy P, Niyogi K, Sengupta DN, Ghosh B (2005) Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H + -ATPase in salt-tolerant and salt-sensitive rice cultivars. Plant Sci 168:583–591

Roychoudhury A, Basu S, Sarkar SN, Sengupta DN (2008) Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. Plant Cell Rep 27:1395–1410

Roychoudhury A, Basu S, Sengupta DN (2011) Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of Indica rice differing in their level of salt tolerance. J Plant Physiol 168:317–328

Saha J, Giri K (2017) Molecular phylogenomic study and the role of exogenous spermidine in the metabolic adjustment of endogenous polyamine in two rice cultivars under salt stress. Gene 609: 88–103

Sang QQ, Shan X, An YH, Shu S, Sun J, Guo SR (2017) Proteomic analysis reveals the positive effect of exogenous spermidine in tomato seedlings' response to high-temperature stress. Front Plant Sci 8:120

Santa-Cruz A, Acosta M, Perez-Alfocea F, Bolarin MC (1997) Changes in free polyamine levels induced by salt stress in leaves of cultivated and wild tomato species. Physiol Plant **101**:341–346

Sarjala T, Kaunisto S (2002) Potassium nutrition and free polyamines of *Betula pendula* Roth and *Betula pubescens* Ehrh. Plant and Soil 238:141–149

Schraudner M, Trost M, Kerner K, Heller W, Leonardi S, Langebartels C, Sanderman H Jr (1990) Ozone induction and function of polyamines in ozone-tolerant and ozone-sensitive tobacco cultivars. In: Flores HE, Arteca RN, Shannon JC (eds) Polyamines and ethylene: biochemistry, physiology and interactions. American Society of Plant Physiologists, Rockville, pp 394–396

Šebela M, Radová A, Angelini R, Tavladoraki P, Frébort I, Pec P (2001) FAD-containing polyamine oxidases: a timely challenge for researchers in biochemistry and physiology of plants. Plant Sci 160:197–207

Serafini-Fracassini D, Sandro AD, Duca SD (2010) Spermine delays leaf senescence in *Lactuca sativa* and prevents the decay of chloroplast photosystems. Plant Physiol Biochem 48:602–611

Shen W, Nada K, Tachibana S (2000) Involvement of polyamines in the chilling tolerance of cucumber cultivars. Plant Physiol 124:431

Shi H, Chan Z (2014) Improvement of plant abiotic stress tolerance through modulation of the polyamine pathway. J Integr Plant Biol 56:114–121

Simões ADN, Diniz NB, Vieira MRDS, Al E (2018) Impact of GA3 and spermine on postharvest quality of anthurium cut flowers (*Anthurium andraeanum*) cv. Arizona Sci Horticult 241:178–186

Slocum RD (1991) Tissue and subcellular localisation of polyamines and enzymes of polyamine metabolism. In: Slocum RD, Flores HE (eds) Biochemistry and physiology of polyamines in plants. CRC Press, Boca Raton, pp 93–105

Sobieszczuk-Nowicka E (2017) Polyamine catabolism adds fuel to leaf senescence. Amino Acids 49:49–56

Szafranska K, Cvikrova M, Kowalska U, Gorecka K, Gorecki R, Martincova O, Janas KM (2011) Influence of copper ions on growth, lipid peroxidation, and proline and polyamines content in carrot rosettes obtained from anther culture. Acta Physiologiae Plant 33:851–859

Sziderics AH, Oufir M, Trognitz F, Kopecky D, Matušikova I, Hausman JF, Wilhelm E (2010) Organ-specific defence strategies of pepper (*Capsicum annuum* L.) during early phase of water deficit. Plant Cell Rep 29:295–305

Tabor CW, Tabor H (1984) Polyamines. Annu Rev Biochem 53:749–790

Takahashi H, Imamura T, Miyagi A, Uchimiya H (2012) Comparative metabolomics of developmental alterations caused by mineral deficiency during in vitro culture of *Gentiana triflora*. Metabolomics 8:154–163

Takahashi T, Kakehi J-I (2010) Polyamines: ubiquitous polycations with unique roles in growth and stress responses. Ann Bot 105(1):1–6

Tanaka N et al (2010) The structure of starch can be manipulated by changing the expression levels of starch branching enzyme IIb in rice endosperm. J Plant Biol 2:507–516

Tassoni A, Antognoni F, Bagni N (1996) Polyamine binding to plasma membrane vesicles from zucchini hypocotyls. Plant Physiol 110:817–824

Tiburcio AF, Besford RT, Borrell A, Mace M (1995) Metabolism and function of polyamines during osmotically induced senescence in oat leaves and protoplasts. In: Wallsgrove RM (ed) Amino acids and their derivatives in higher plants. Cambridge University Press, Cambridge, pp 205–225

Tiburcio AF, Kaur-Sawhney R, Galston AW (1990) Polyamine metabolism. In: Miflin BJ, Lea PJ (eds) The biochemistry of plants, intermediary nitrogen fixation. Academic Press, New York, pp 283–325

Todorova D, Moskova I, Sergiev I, Alexieva V, Mapelli S (2008) Changes in endogenous polyamines and some stress markers content induced by drought, 4PU-30 and abscisic acid in wheat plants. In: Khan N, Singh S (eds) Abiotic stress and plant responses. International Publishing House, New Delhi/Bangalore/Mumbai, pp 205–215

Toumi I, Moschou PN, Paschalidis KA, Bouamama B, Salem-Fnayou AB, Ghorbel AW, Mliki A, Roubelakis-Angelakis KA (2010) Abscisic acid signals reorientation of polyamine metabolism to orchestrate stress response via the polyamine exodus pathway in grapevine. J Plant Physiol 167:519–525

Uemura T, Tachihara K, Tomitori H, Kashiwagi K, Igarashi K (2005) Characteristics of the polyamine transporter TPO1 and regulation of its activity and cellular localization by phosphorylation. J Biol Chem 280:9646–9652

Urano K, Hobo T, Shinozaki K (2005) *Arabidopsis* ADC genes involved in polyamine biosynthesis are essential for seed development. FEBS Lett 579:1557–1564

Urano K, Maruyama K, Ogata Y, Morishita Y, Takeda M, Sakurai N et al (2009) Characterization of the ABA-regulated global responses to dehydration in *Arabidopsis* by metabolomics. Plant J 57:1065–1078

Urano K, Yoshiba Y, Nanjo T, Igarashi Y, Seki M, Sekiguchi F, Yamaguchi-Shinozaki K, Shinozaki K (2003) Characterization of *Arabidopsis* genes involved in biosynthesis of polyamines in abiotic stress responses and developmental stages. Plant Cell Environ 26:1917–1926

Vladimir V, Shevyakova N (2007) Polyamines and stress tolerance of plants. Plant Stress 1(1): 50–71

Wang B-Q, Zhang Q-F, Liu J-H, Li G-H (2011) Overexpression of PtADC confers enhanced dehydration and drought tolerance in transgenic tobacco and tomato: effect on ROS elimination. Biochem Biophys Res Commun 413:10–16

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Wen XP, Ban Y, Inoue H, Matsuda N, Moriguchi T (2010) Spermidine levels are implicated in enhanced heavy metal tolerance in a spermidine synthase –overexpressing transgenic European pear by exerting antioxidant activities. Transgenic Res 19:91–103

Wen X-P, Ban Y, Inoue H, Matsuda N, Moriguchi T (2009) Aluminum tolerance in a spermidine synthase-overexpressing transgenic European pear is correlated with the enhanced level of spermidine via alleviating oxidative status. Environ Exp Bot 66:471–478

Wen X-P, Pang X-M, Matsuda N, Kita M, Inoue H, Hao Y-J, Honda C, Moriguchi T (2008) Overexpression of the apple spermidine synthase gene in pear confers multiple abiotic stress tolerance by altering polyamine titers. Transgenic Res 17:251–263

Wimalasekera R, Tebartz F, Scherer GFE (2011) Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses. Plant Sci 181:593–603

Woo HR, Kim HJ, Nam HG, Lim PO (2013) Plant leaf senescence and death–regulation by multiple layers of control and implications for aging in general. J Cell Sci 126:4823–4833

Xu Y, Shi GX, Ding CX, Xu XY (2011) Polyamine metabolism and physiological responses of *Potamogeton crispus* leaves under lead stress. Russian J Plant Physiol 58(3):460–466

Yamaguchi K, Takahashi I, Berberich T, Imai A, Miyazaki A, Takahashi T, Michael A, Kusano T (2006) The polyamine spermine protect against high salt stress in *Arabidopsis thaliana*. FEBS Lett 580:6783–6788

Yamaguchi K, Takahashi Y, Berberich T, Imai A, Takahashi T, Michael AJ, Kusano T (2007) A protective role for the polyamine spermine against drought stress in *Arabidopsis*. Biochem Biophys Res Commun 352:486–490

Yang C, He S (2001) The relationship between polyamine and membrane lipid peroxidase during the senescence of cut rose flowers. Acta Botanica Boreali Occidentalia Sinica 21:1157–1161

Yang H, Shi G, Wang H, Xu Q (2010) Involvement of polyamines in adaptation of *Potamogeton crispus* L. to cadmium stress. Aquat Toxicol 100:282–288

Yang J, Zhang J, Liu K, Wang Z, Liu L (2007) Involvement of polyamines in the drought resistance of rice. J Exp Bot 58:1545–1555

Ye XS, Avdiushko SA, Kuc J (1994) Effect of polyamines on in vitro phosphorylation of soluble and plasma membrane proteins in tobacco, cucumber and *Arabidopsis thaliana*. Plant Sci 97: 109–118

Zacchini M, Iori V, Mugnozza GS, Pietrini F, Massacci A (2011) Cadmium accumulation and tolerance in *Populus nigra* and *Salix alba*. Biologia Plantarum 55(2):383–386

Zeid IM (2004) Response of bean (*Phaseolus vulgaris*) to exogenous putrescine treatment under salinity stress. Pak J Biol Sci 7(2):219–225

Zhang Z-K, Liu S-Q, Hao S-S, Liu S-H (2010) Grafting increases the copper tolerance of cucumber seedlings by improvement of polyamine contents and enhancement of antioxidant enzymes activity. Agric Sci China 9(7):985–994

Zhao J, Shi G, Qiuhong Y (2008) Polyamines content and physiological and biochemical responses to ladder concentration of nickel stress in *Hydrocharis dubia* (Bl.) Backer leaves. Biometals 21: 665–674

Zhou Q, Yu B (2010) Changes in content of free, conjugated and bound polyamines and osmotic adjustment in adaptation of vetiver grass to water deficit. Plant Physiol Biochem 48:417–425